Reference: *Biol. Bull.* **151**: 587–600. (December, 1976)

EXCHANGES OF SODIUM AND CHLORIDE AT LOW SALINITIES BY *NEREIS DIVERSICOLOR* (ANNELIDA, POLYCHAETA)

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Recent studies on the uptake and exchanges of water and the principal inorganic ions in the well-known brackish-water polychaete *Nereis diversicolor* (F. O. Müller) indicate that this worm employs a number of physiological mechanisms in its osmotic and ionic regulation (Oglesby, 1970, 1972; Smith 1970a, b, c; Fletcher, 1970, 1974a, b, c; review by Oglesby, 1969a), but there remain many unanswered questions regarding the inter-relationships of these processes and of their control. One area of interest is that of the relationship between the regulation of the two major ions, sodium and chloride. Smith (1970a, b, c) as well as Jørgensen and Dales (1957) and Oglesby (1969b) has postulated an active uptake of chloride, without specifying whether chloride as such is being transported, or moved as a consequence of or in relation to the transport of some other ion, such as sodium. Jørgensen and Dales postulated reductions in permeability of the body wall both to water and chloride, but Smith (1970a, b) demonstrated a reduction in apparent permeability to water without finding it necessary to invoke a reduction in permeability to chloride. Jørgensen and Dales suggested that the urine of *N. diversicolor* could be iso-ionic in respect to chloride; Smith (1970c) demonstrated hypo-osmotic urine. Oglesby (1970, 1972) has provided much detailed evidence on the steady-state levels of water, chloride, sodium and potassium, and especially on the efflux of sodium; he has found an active uptake of sodium as well as a reduction in body-wall permeability to it at low salinities and gave evidence that potassium is not regulated in the body fluid. Fletcher (1970) studied the relationship of the inside-negative body wall potential to external salinity, as well as the regulation of calcium and magnesium, and the control of body volume (1974a, b, c).

The present study includes a comparison of the patterns of sodium and chloride exchange rates as functions of salinity in the steady state and an attempt to determine whether or not the sodium uptake of *N. diversicolor* is activated at low salinities. The assumptions and conclusions involved in the chloride balance-sheet presented by Smith (1970a, b) are reinvestigated and are modified in the light of fresh data.

Materials and Methods

Basic methods

Nereis diversicolor was collected in shallow water of Vellerup Vig, a small bay of the Danish Isefjord, at a site where salinity is markedly, although variably,

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lowered by inflow of a freshwater stream. This site was the source of worms used by Smith (1955a), Jørgensen and Dales (1957), and Ahearn and Gomme (1975). The biology of *N. diversicolor* in the Isefjord has been discussed in detail by Rasmussen (1973). After collection, worms were transported to Copenhagen in undiluted Isefjord water (*ca.* 65 per cent sea water, % SW), and sorted in the laboratory into large plastic boxes provided with numerous short lengths of glass tubing, in which worms took up residence within a few hours. Worms were maintained with aeration at 14° C in various dilutions of artificial sea water made up according to Hale (1958). "100% SW" as used in these studies had a sodium concentration of 470 mM/liter and a chloride concentration of 550 mM/liter. It was diluted with distilled water for concentrations down to 5% SW. Below 5% SW, dilutions were with a solution resembling hard pond water (Smith, 1970a), but lacking Na and Cl. This dilutant contained Ca^{++}, 1.14 mM liter; Mg^{++}, 0.39 mM/liter; K^{+}, 0.11 mM/liter, and was made up at 10× final strength. K was added as $KHCO_3$ or KNO_3, Mg as $MgSO_4$, and Ca as $Ca(OH)_2$; the cloudy alkaline fluid resulting was cleared by *ca.* 25 drops of conc. H_2SO_4 per liter to a final pH of 5.5 or 6. The product could not be told by taste from distilled water, and permitted good survival in SW dilutions down to 0.2% SW (ca. 0.9 mM Cl/liter). There was survival in 0.1% SW so diluted but, as the worms were noticeably sluggish and were sticky from excess mucus, they were not used experimentally. For simplicity, dilutions of sea water are expressed in the text as the percentage of sea water (% SW), since a given dilution of SW has different molarities of Na and Cl.

Adaptations to various salinities were made stepwise over periods of one to several days, and worms remained at the final adaptational salinity for 4–10 days before being used in experiments. In the standard pattern of experiment, used for most ^{22}Na and all ^{36}Cl exchange studies, worms were exposed individually for 1 hour in 10 ml of radioactive medium at 15° C. In any single experiment 16 worms were used, and sub-groups of 5–8 worms were tested at different salinities at the same time, these groupings being arranged so as to cancel out progressive or serial changes resulting from different lengths of residence under laboratory conditions which might impose some trend upon the results of studies made on successive days.

^{22}Na-uptake. ^{22}Na was obtained from the Radiochemical Centre, Amersham, England as neutral NaCl. Media were measured into vials in a water-bath at 15° C. For each experiment, worms were visually sorted into approximately equal size groups, weighed after blotting on filter paper, and dropped individually into a series of vials of 15 ml of adaptational medium at 15° C, this medium being the same as that for the radioactive tracer to be used next. This passage through an intermediate inactive medium served to provide a period of adjustment and a comparable activation for all worms, since it is known (Smith, 1970a) that worms so transferred between identical media initially show a net loss of chloride and of weight as a result of the expulsion of urine with the increased activity. Starting 30–60 minutes after the transfer to adaptational medium, worms were blotted and transferred at 2-minute intervals to 10 ml of medium containing the tracer, still at 15° C. After one hour of undisturbed exposure, worms were individually blotted, rinsed in a fresh sample of inactive medium for 2–4 seconds, re-blotted,

and dropped into 2 ml of 4% formaldehyde previously measured into a series of polyethylene counting vials and tightly stoppered. Samples were counted to a constant 10,000 counts on a "Selektronic" solid state (NaI) scintillation gamma counter with sample changer and printout. Four 10 μl portions of unused radioactive medium of each salinity employed, as well as a number of blanks for background, were also counted in each experiment.

^{36}Cl-uptake. ^{36}Cl was obtained as neutral NaCl from the Danish Atomic Energy Commission Research Center, Risø, Denmark. Worms were handled as above, up to the point of removal from the one-hour exposure in radioactive medium, blotting, rinsing, and re-blotting. In ^{36}Cl-exchange measurements, worms were then dropped individually into a previously measured 2 ml of 3 N HNO_3 in tightly-capped resistant polyethylene centrifuge tubes and digested for 48 hr at room temperature, with two or three vigorous mechanical shakings to ensure complete disruption, centrifuged, and a 1 ml sample of clear supernatant transferred to a capped plastic scintillation vial, to which 10 ml of scintillation fluid was later added (one liter toluol, DDH, sulfur-free, Merck; 550 ml Triton-X; 5 g PPO; 200 mg POPOP). One ml of the digesting acid was added to each of a number of vials for background counts, and 10 μl of each active medium was added to one ml of acid, in triplicate, for determination of its activity.

Sodium concentration. Sodium was measured by means of an Eppendorf Flame Photometer (Netheler and Hinz GMBH, Hamburg), employing NaCl standards diluted 1 : 500 in 1.5 mM/liter KNO_3 to counteract potassium interference. Samples of media, coelomic fluid, and the acid ^{36}Cl extracts were diluted in this medium also, usually 1 : 500, although with media of 1% SW, 50 μl were added to 5 ml, and of 0.2% SW, 100 μl had to be used.

Chloride concentration. Chloride was measured by use of a model CMT10 Chloride Titrator (Radiometer, Copenhagen), employing NaCl standards from the same stock used for sodium, but diluted 1 : 100 in the same KNO_3 dilutant. Appropriate dilutions were made of media, coelomic fluid, and the acid ^{36}Cl extracts, namely, 1 : 100 for coelomic fluid samples and for media of 20% SW and stronger, 1 : 20 for 10% SW, and full strength for media of 5% SW or lower concentration. In the latter instances, the volume titrated could be varied by use of appropriate Petersen constriction pipettes.

Experimental procedures

Pattern of steady-state sodium exchange as a function of salinity. The pattern of steady-state sodium exchange in *Nereis diversicolor* has been thoroughly investigated by Oglesby (1970, 1972) in studies focused upon sodium efflux in worms from an estuarine habitat in northern England. In the present study of worms from the more stable intermediate salinities of the Isefjord, comparative data were obtained; these will be seen to be in line with Oglesby's results, although exact correspondence was neither obtained nor expected. In the initial series of experiments, worms were exposed for different lengths of time (0.5 to 4 hr) to radioactive media containing ^{22}Na, and interpolation was used to obtain a rate of exchange for one hour. This method proved cumbersome, so that a standard one-hour exposure was adopted in subsequent work with ^{22}Na and for all work with ^{36}Cl.

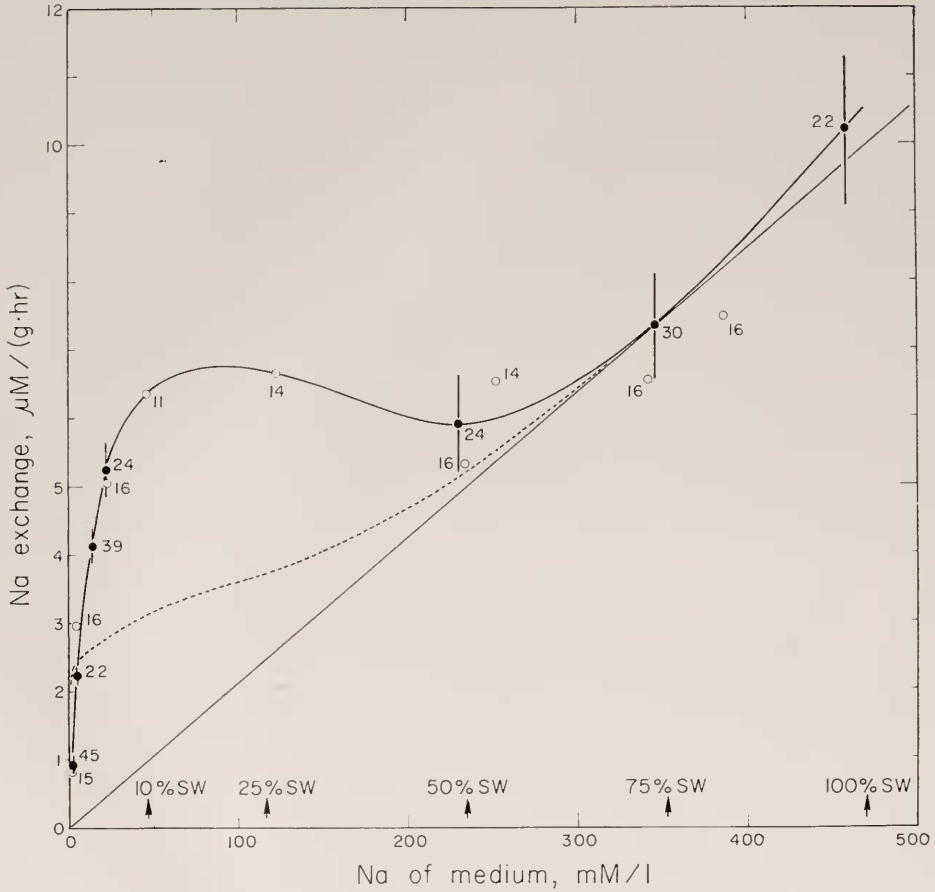

FIGURE 1. Steady-state Na-exchange in *N. diversicolor* (as influx of ^{22}Na into whole body) as a function of external concentration. Closed circles and solid curve calculated from one-hour exposures; open circles by interpolation to one hour; number of worms (n) is shown by each point. In this and following figures, vertical bars show ± 2 s.e. Dotted curve shows passive integumental diffusional efflux, proportional to coelomic Na-concentration (determined on 98 worms), calculated on the assumption that Na-permeability of body wall does not change with salinity. Diagonal straight line shows passive diffusional influx, proportional to external Na-concentration, also assuming no change in Na-permeability, and assuming that total exchange in 75% SW is passive.

Pattern of steady-state chloride exchange as a function of salinity. The rate of Cl-uptake or steady-state exchange in *N. diversicolor* as a function of the external chloride concentration has been shown by Jørgensen and Dales (1957) and Smith (1970a) to have a characteristic pattern with the exchange proportional to external chloride concentration in salinities greater than 50% SW, a more or less constant level over the intermediate range of salinities, a tendency to show a peak of exchange in low salinities (± 10% SW), and a marked reduction in very low salinities (down to 0.4% SW, = *ca.* 2.5 mM Cl/liter). Smith (1970a) constructed a balance-sheet for chloride exchanges, using the assumptions of Potts and

Parry (1964), by which it was possible to conclude that *N. diversicolor* could maintain chloride balance at an external chloride concentration as low as 2.5 mM/liter by utilizing the observed rate of Cl-uptake and the presumed active recovery of chloride from the consequently hypotonic urine, *without the necessity of postulating a reduction in the permeability of the body wall to chloride,* as had Jørgensen and Dales. The inferences drawn from the balance-sheet were supported by the demonstration (Smith, 1970b) of a reduction in the apparent permeability to water of the body wall (resulting in the reduction of urine volume), and by the direct demonstration (Smith, 1970c) that the urine of *N. diversicolor* is hypo-osmotic to coelomic fluid at low salinities.

However, the chloride balance-sheet used by Potts and Parry (1964) and by Smith (1970a) involves acceptance of two questionable assumptions, namely, that the electrical potential across the body wall may be ignored, and that the permeability to diffusional (passive) exchange of chloride does not change with salinity. But in view of the evidence for such a change presented by Jørgensen and Dales (1957), and the fact that Oglesby (1970) demonstrated a reduction in the permeability of the body wall to sodium (confirmed in the present study), it is necessary to re-examine the assumption of no chloride-permeability change. That Smith (1970a) found it unnecessary to invoke such a permeability change was because, in his study, the total Cl-uptake (= total efflux) was, at all salinities down to 0.4% SW, greater than the calculated passive or diffusional integumental output of chloride. The purpose of the present work was to re-study chloride uptake at even lower salinities, in order to test whether or not a Cl-permeability change must be invoked. Secondly, the intention was to compare the patterns of sodium and chloride exchange at low salinities to see if there are indications that the course of Cl-uptake is independent of Na-uptake, or is in some way coupled to or dependent upon Na-uptake.

Test for activation of Na-uptake mechanism at low salinities. It has been shown in gammarid crustaceans (Shaw and Sutcliffe, 1961) that adaptation to very low environmental Na-concentrations results in an activation of the sodium uptake mechanism. This was shown by the fact that, when gammarids adapted to a very low sodium concentration were transferred to a higher concentration, the rate of uptake of sodium exceeded that which would be expected of animals in steady-state adaptation to the higher concentration. It is of interest to determine if a comparable response is shown by *Nereis diversicolor.* Worms were adapted for five or more days at 15° C to salinities of *ca.* 0.2, 1, 3, and 5% SW (Na-concentrations of *ca.* 1.1, 4.6, 13.6, and 22.3 mM/liter, respectively). Uptake of ^{22}Na was then measured in a one-hour exposure, in the following groupings:

Adapted in low Na ⟶ tested in low Na

Adapted in low Na ⟶ tested in higher Na

Adapted in higher Na ⟶ tested in higher Na

In any single experiment, involving 16 worms, groups of 5–6 individuals representing all three transfer classes were tested simultaneously in order to compensate for any serial or progressive change in behavior resulting from increasingly long periods of laboratory adaptation, or from factors which might vary from

day to day. The uptakes recorded in symmetrical tranfers (adaptational and test media the same) formed the basis for the "steady state" uptake curve in Figure 4, with which the results of asymmetrical transfer (from a low to a higher Na-concentration) are compared. "Low" Na-concentrations were 0.2 and 1% SW; "higher" concentrations were 1, 3, and 5% SW in these experiments. An activation of the Na-uptake mechanism should result in the "transfer" curve lying above the "steady-state" curve, if other factors remained the same.

Results

Pattern of steady-state sodium exchange as a function of salinity

As shown in Figure 1, the results of interpolation to one hour were in general agreement with the values for total Na-influx obtained for one-hour exposures upon which the uptake curve is drawn. This curve shows several characteristics of the steady-state exchange of sodium (in the steady state the total Na-influx equals the total Na-efflux). First, at higher salinities (75–100% SW) the exchange of sodium is proportional to external and internal (coelomic fluid) Na-concentrations, as would be expected in the range of ionic conformity if no active transport were taking place. However, in the 50–75% SW range, where ionic

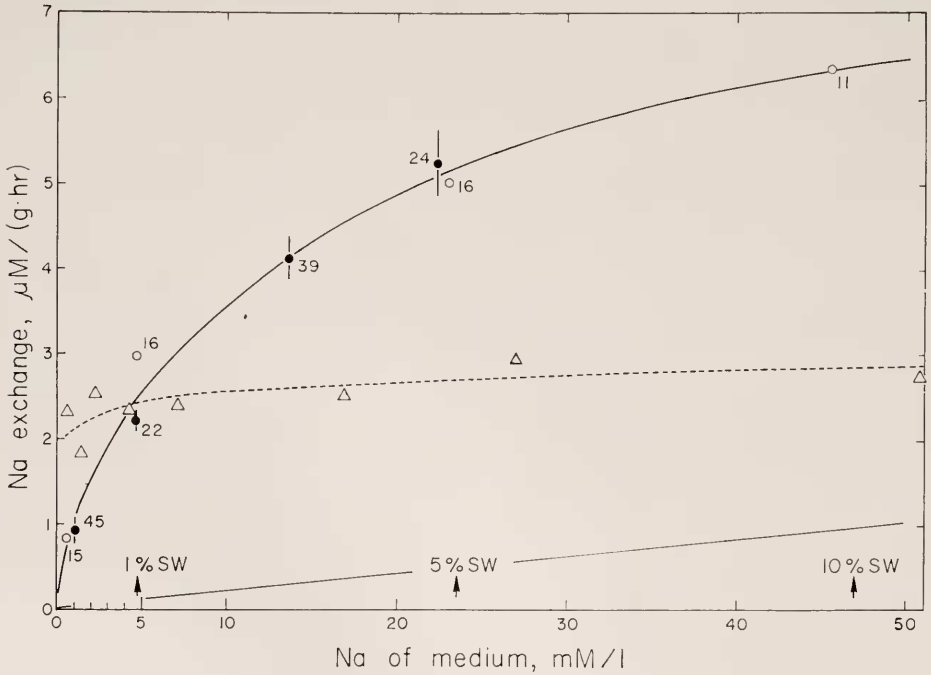

Figure 2. Expansion of Na-exchange curves of Figure 1 for Na-concentration range below 50 mM/liter. Symbols as in Figure 1, with addition of triangles representing passive diffusional efflux values, calculated from coelomic fluid Na-concentrations, on which dotted passive efflux curve is based.

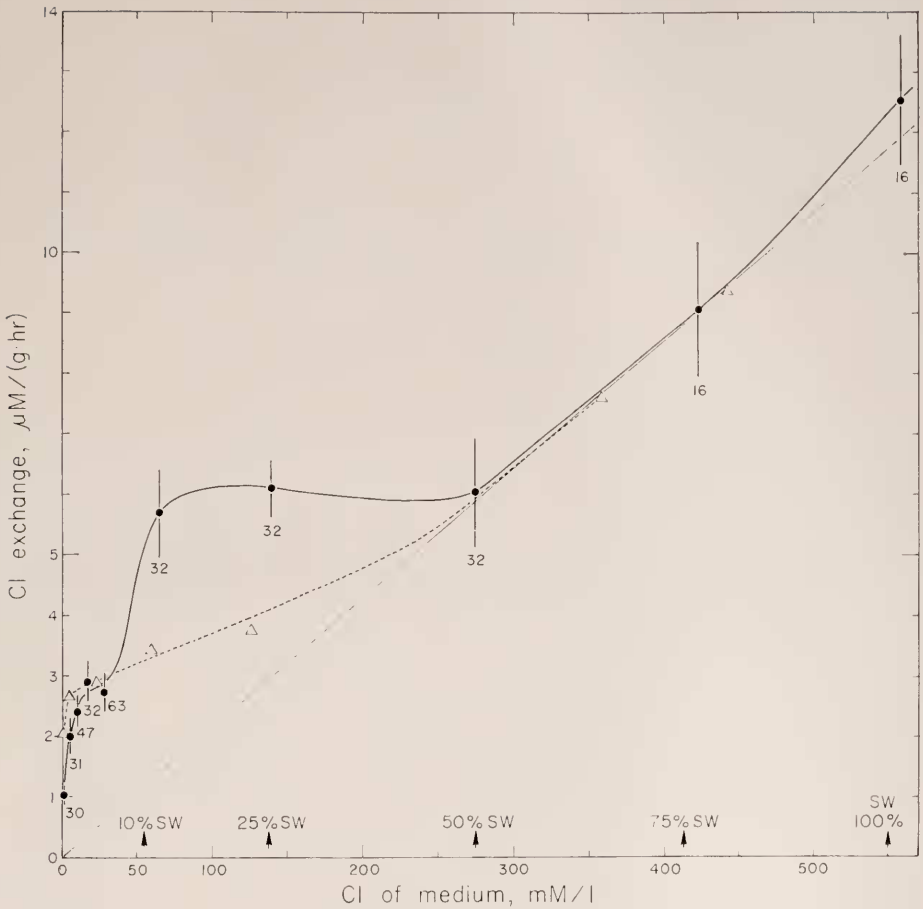

FIGURE 3. Steady-state Cl-exchange in *N. diversicolor* (as influx of ^{36}Cl into whole body) as a function of external concentration. Closed circles and solid curve indicate Cl-influx; triangles and dotted curve show integumental diffusional efflux, proportional to coelomic Cl-concentration (determined on 101 worms), calculated on assumption of no change in Cl-permeability of body wall. Diagonal straight line shows passive diffusional influx, proportional to external Cl-concentration, also assuming no change in Cl-permeability, and assuming that total exchange in 75% SW is passive.

conformity also prevails, the exchange is somewhat elevated, suggesting some active transport process. In Figures 1 and 2 the straight diagonal line represents the *passive influx* that would be expected on the basis of external Na-concentration alone, assuming no change in Na-permeability of the body wall. The dotted curve in Figures 1 and 2 represents the *passive efflux* expected on the basis of internal (coelomic) Na-concentration alone, again assuming no change in body-wall Na-permeability. Secondly, below 50% SW, the measured rate of Na exchange (equal to total influx in the steady state) is much elevated above the level of passive efflux, indicating nondiffusional (urinary) loss of sodium, as well as

active inward transport process(es). There is a tendency for Na-exchange to be maximal in *ca.* 20% SW or below, where urinary output and inward sodium transport are presumed to be high. Thirdly, there is a very marked exchange at low external Na-concentrations; if a value of *ca.* 6.5 μM Na/(g·hr) represents the saturation level of the system, then the external concentration necessary for half the maximum uptake rate is *ca.* 10 mM/liter. The exchange rate rises rapidly with increased availability of sodium, to level off at 6–7 μM Na/(g·hr). But at the lowest external Na-concentrations, below ca. 6 mM/liter, the measured Na-exchange falls below the diffusional efflux of sodium that would be calculated if Na-permeability were constant at all salinities (Figs. 1 and 2).

Pattern of steady-state chloride exchange as a function of salinity

Chloride influx in the present experiments has the pattern shown in Figure 3. As in the earlier study (Smith, 1970a), there is proportionality of uptake to Cl-concentration in the range of 50–100% SW, where coelomic and external Cl-concentrations are nearly equal. Over the range from *ca.* 10 to 50% SW the influx is fairly constant, and lies well above the calculated integumental diffusional efflux (dotted curve), suggesting that in this range of ionic and osmotic regulation there must be a considerable nonintegumentary (probably urinary) loss of chloride as well as active inward transport of chloride. The peak of Cl-exchange indicated in *ca.* 10% SW by Jørgensen and Dales (1957) and by Smith (1970a) is not evident in the present data but, it may be noted, such a peak was not obtained in the previous shorter-term (4 hr) experiments (Smith, 1970a). Below 10% SW, the uptake curve drops markedly, and clearly lies below the dotted curve of integumental diffusional output in the salinity range from 5% SW down to *ca.* 0.2% SW [Cl. *ca.* 1 mM/liter; half the lowest value tested by Smith (1970a)]. In this low-salinity range, the chloride-exchange curve is depressed below that for sodium (Fig. 1), leveling off in the range from 2 to 5% SW and then resuming its rise to a plateau level of *ca.* 6 μM Cl/g·hr). This level of chloride uptake, measured as uptake of the whole body, is consistent with the uptake plateau of *ca.* 8 μM/(g·hr) recorded by Smith (1970a) in the shorter-term (4 hr) exposures, calculated on the basis of activities obtained in coelomic fluid. Such determinations were misleadingly high, and resulted from the oversimplification of regarding *N. diversicolor* as a single compartment of coelomic fluid surrounded by a body wall. The present results differ most markedly from the earlier results in that the total Cl-exchange in the salinity range below 8–10% SW is too low to maintain the steady state in the face of a diffusional integumental efflux calculated on the assumption of no change in diffusional permeability to chloride, hence the latter assumption is untenable. Further, the depression of the chloride exchange curve in the 1–5% SW range is in sharp contrast to the very steep and uninterrupted rise of the sodium exchange curve in this low salinity range (Fig. 1).

Test for activation of Na-uptake mechanism at low salinities

The results of these experiments are in general insufficient to support the hypothesis of an activation of the sodium uptake mechanism of *N. diversicolor*

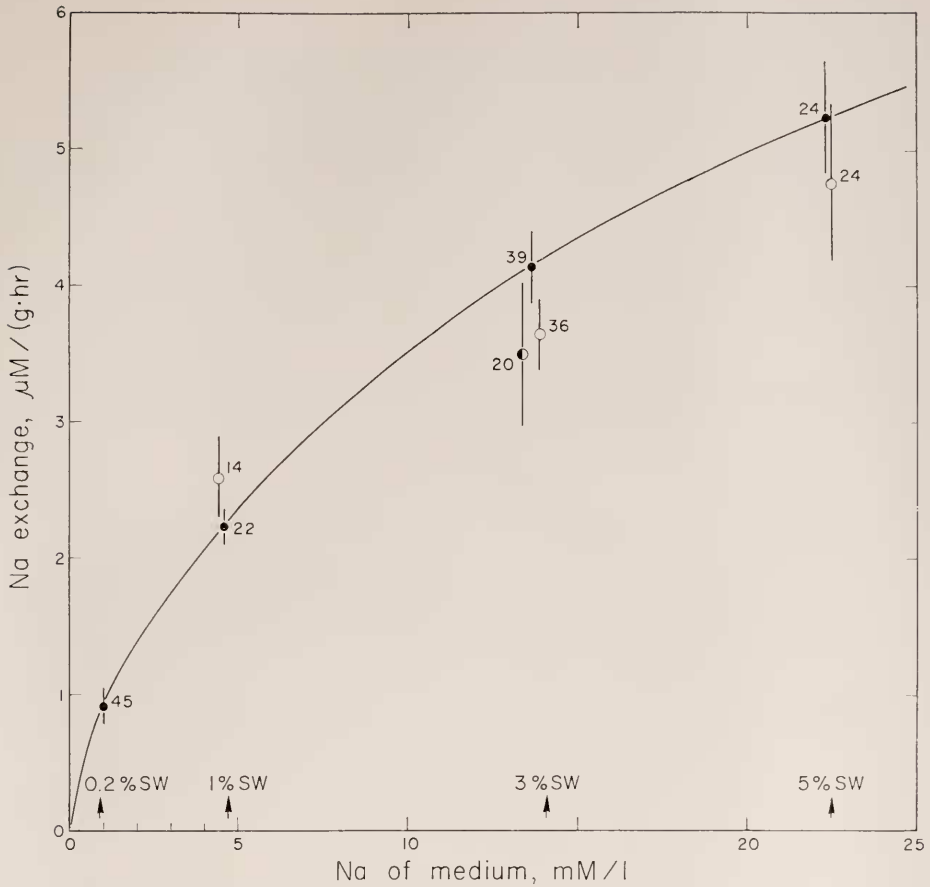

FIGURE 4. Effect upon Na exchange-rate of adaptation to very low Na-concentrations followed by abrupt transfer to higher concentrations. Closed circles and curve indicate Na-influx of control worms in steady state adaptation; open circles show Na-influx after transfer from 0.2% SW (Na = *ca.* 1 mM/liter), half-open circle after transfer from 1% SW (Na = *ca.* 5 mM/liter).

at low salinities. As is indicated in Figure 4, only in the case of transfer from 0.2% SW to 1% SW is there a scarcely significant increase of Na-uptake over the rate characteristic of worms adapted to 1% SW. In all other types of transfer: from 0.2% SW to 3% or 5% SW, and from 1% to 3% SW, the uptake as measured in the first hour after transfer tended to fall below the steady-state curve. Although the rates of the several transfer groups are not statistically significantly lower, they are generally below the steady-state curve and (with the possible exception of the 0.2 to 1% SW transfer) indicate no activation of Na-uptake. The combined results of transfer from 0.2 and 1% SW to 3% SW are significantly lower statistically than the steady-state uptake rate of animals adapted to 3% SW.

Discussion

The pattern of sodium-exchange as a function of external concentration (Fig. 1) suggests that the uptake mechanism for sodium operates with a high affinity for that ion, down to external concentrations of 1 mM/liter or less, at which the uptake rate is very low [$< 1\ \mu M/(g \cdot hr)$]. It must follow, as Oglesby (1972) has already shown, that *N. diversicolor,* in adapting itself to salinities of 1–2% SW, must utilize a lowering of body wall permeability to sodium as part of its adaptational repertoire. Otherwise, outward diffusion of Na from the high concentration maintained in the body fluid would exceed the measured steady-state influx. The dotted curve in Figure 1, shown in more detail in Figure 2, represents the integumental diffusional efflux calculated *on the assumption* that the permeability to sodium does not change and that outward diffusion of sodium is proportional to the concentration of Na in coelomic or extracellular fluids. At external Na-concentrations below *ca.* 5 mM/liter (*ca.* 1% SW), the sodium influx could not balance the efflux, hence the assumption of a constant diffusional sodium-permeability cannot be supported. In contrast to the pattern of Cl-exchange (Fig. 3), there is little indication of proportionality of Na-exchange to external salinity in the 50–75% SW range as might be expected *on the assumption* that only passive diffusional exchange takes place in the range of osmotic and ionic conformity, and by analogy with the evidence for chloride. The straight line in Figure 1, drawn on the assumption that exchange is proportional to external Na-concentration in 75% SW and higher salinities, lies sufficiently below the Na-exchange curve as to suggest that it is not necessary to assume that the active uptake mechanism for sodium is simply shut off when a state of conformity is reached. The present results suggest, rather, that some degree of active inward transport of sodium, with some form of coupled outward transport (possibly exchange diffusion) continues to operate in the 50–75% SW range in steady-state conformity. Oglesby, however, (1972) found exchange diffusion of Na only in salinities below 25% SW, and the matter needs further examination.

The pattern of chloride exchange (Fig. 3) differs in certain details, both from the earlier results obtained by Smith (1970a), and from the pattern of sodium exchange obtained in the present study (Fig. 1), so as to indicate that the active inward transport of sodium and chloride are by independent processes. Chloride exchange rates as determined in England by Smith (1970a) were based upon ^{36}Cl-activity measured in coelomic fluid, and in consequence appear higher than the values obtained in the present study of uptake by whole worms (in which the intracellular compartment, low in chloride, is included). In Smith's (1970a) studies, *N. diversicolor* was treated as a single compartment of coelomic fluid within a body wall, the Cl-permeability of which was assumed to be unchanged at lower salinities. The calculation of an integumental diffusional efflux *on the assumption that outward diffusional permeability remained constant* was done in order to permit discussion of the treatment of water and chloride fluxes in *N. diversicolor* in the terms used by Potts and Parry (1964, pp. 145–152). The resulting balance sheet for chloride fluxes proved useful at the time, but it is now apparent that it was based on certain untenable assumptions, and must be set aside. The assumption that the outward diffusional permeability to chloride is constant is discredited by the present results because, as in the case of sodium,

the uptake rate of chloride at very low salinities in these experiments proves inadequate to maintain the steady state without reduction of the calculated diffusional efflux, shown by the dotted curve in Figure 3. It is no longer possible, by such a simple balance-sheet as was used by Smith (1970a) to fractionate the steady-state Cl-efflux between an "integumental" and a "urinary" component. It can be concluded that *N. diversicolor,* in maintaining chloride balance at very low salinities, does utilize Cl-permeability reduction, as postulated by Jørgensen and Dales (1957), together with a reduction of apparent water-permeability and the active inward transport of chloride both from the medium and from the consequently hypotonic urine. The previous neat and simple balance sheet is, however, inadequate to depict the chloride exchanges at low salinities, and must, in that respect at least, be discarded.

The pattern of Cl-exchange (Fig. 3) further differs from the previous (Smith, 1970a) exchange curve for this ion, as well as from the Na-exchange curve (Fig. 1), in showing a depression in the Cl-concentration range below 50 mM/liter (*ca.* 10% SW). At least four hypotheses to account for such a depression might be considered. First, there might be a physiological difference between *N. diversicolor* populations of British estuaries and those of the more stable intermediate salinities of the Danish Isefjord. There is no factual basis for this hypothesis; such evidence as exists from studies employing identical methods on British and Danish *N. diversicolor* (Smith, 1955b) suggest similarity rather than differences in the regulation of chloride. Secondly, there might be some as-yet-undetected technical or experimental flaw. This is a possibility difficult to prove or disprove. The 1970a study employed planchet counting of ^{36}Cl in coelomic fluid samples after 4 and 18 hour exposures; the present study used scintillation counting of acid extracts of whole worms after one hour exposures, but there is no reason why such differences in method should alter the shape of the Cl-exchange curve. After discounting the above hypotheses, two physiological hypotheses may be considered.

The first of these is that there might be two different chloride uptake mechanisms involved. One, operating at very low Cl-concentrations, might have a high affinity for chloride and a low capacity for transport; it would have a concentration for half-maximal rate of uptake of about 4 mM Cl/liter and would be essentially saturated at an external Cl-concentration of *ca.* 20 mM/liter, with a maximal uptake rate of *ca.* 3 μM Cl/(g·hr). The second, operating over a wider salinity range, would have lower affinity but a higher capacity for chloride; it would be saturated at an external Cl-concentration of *ca.* 75 mM/liter, with an uptake rate approaching 6 μm Cl/(g·hr), and would attain half-maximal uptake rate at a concentration of 30–40 mM Cl/liter. By contrast, the sodium uptake curve suggests a single mechanism, with a concentration necessary for half the maximal uptake rate of *ca.* 8–10 mM Na/liter, and becoming saturated at a concentration of *ca.* 40–50 mM Na/liter, at a rate of 6–7 μm Na/(g·hr). Curves illustrating such a two-mechanism hypothesis could easily be drawn, but the only real difficulty is that, except for the present curve (Figure 3), there is not a shred of evidence for it.

The remaining physiological hypothesis is that Cl-uptake below 10% SW might be depressed by or related to the opposing, inside-negative, body-wall potential

(Smith, 1970a; Fletcher, 1970). Were this potential linearly related to external salinity, no such localized depression in the exchange curve would be expected, but Fletcher's data show that the inside-negative potential is negligible down to the low external Cl-concentration of *ca.* 50–60 mM Cl/liter (10% SW), and then rises to nearly 50 mV inside-negative as external Cl-concentrations fall to 1 mM Cl/liter (*ca.* 0.2% SW). This range of rising potential corresponds exactly to the range in which the depression of the Cl-exchange curve is seen. Fletcher's data are thus compatible with the idea that the body wall potential has an important relationship to the uptake of chloride by *N. diversicolor* at very low salinities. Possibly the inside-negative potential is itself the result of the active inward transport of chloride, the uptake of sodium being incapable of producing such a potential.

Activation of the Na-uptake mechanism of *N. diversicolor* at low salinities has not been demonstrated, but this failure to show activation of Na-uptake does not prove its absence. The assumption that the method used might show an activation of uptake rested upon the prior assumption that other factors, such as permeability to the passage of Na, are not greatly altered. However, the latter assumption is not tenable because the permeability of the body wall to sodium is decreased at low salinities (Oglesby, 1970; this study). If, in adapting to 0.2% SW, *N. diversicolor* establishes a permeability to Na sufficiently lower than that in 3 or 5% SW, then the return to a higher salinity might find it unable to achieve the rate of inward transport expected at that higher salinity. The result, in terms of Na-influx, would be the resultant of any activation of the Na-uptake mechanism together with any reduction of Na-movement resulting from decreased permeability. The results of these experiments are compatible with the hypothesis that a sodium-permeability lowering in very low salinities (*e.g.*, in 0.2 and 1% SW), is sufficiently long-lasting to depress the uptake in 3 and 5% SW for at least the hour of the test in these experiments. The slight tendency for an elevation of uptake in the transfer from 0.2 to 1% SW might suggest an activation of uptake, with a small enough difference in sodium permeability to permit the activation to be revealed. Thus, while the overall result of a depressed Na-uptake after transfer from a very low to a higher salinity seems best explained by Na-permeability reduction as the principal adaptation to the very low salinity, the possibility of activation of the Na-uptake mechanism is not excluded. The decreased permeability characterizing adaptation to extremely low salinities limits the uptake to less than what the transport system could carry when more sodium is suddenly made available, and must tend to mask any activation of uptake. Experiments of this type cannot, without additional information, distinguish between absence of activation and the masking of adaptation by altered permeability.

In possible contrast to these results, Oglesby (1972) has reported an augmentation of Na-exchange after transfer of *N. diversicolor* from short exposures in deionized water back to adaptational salinities of 25% SW or higher. This phenomenon does not appear comparable to the present experimental results. However, when Oglesby exposed worms adapted in 0.5–6% SW to deionized water for 2–4 hr and then returned them to their adaptational media, he found that they resumed their characteristic efflux constant immediately. They thus seem to show

considerable stability in respect to variation in the low-salinity range, and further study is needed to clarify the matter.

These studies were carried out at Zoophysiological Laboratory A, August Krogh Institute, University of Copenhagen, Denmark. I am indebted to Professor C. Barker Jørgensen and Lecturer Jørgen Gomme for much help, cooperation, and stimulating discussion. I thank Drs. Lars Ole Simonsen of Zoophysiological Laboratory B for permission to use flame photometer and chloride titrator. Partial support of the research by a NATO Senior Fellowship and by the Committee on Research, Berkeley Campus, of the University of California is gratefully acknowledged, as is also the housing provided by Danmarks Nationalbank.

Summary

1. Experiments to compare the exchange (total influx) of sodium and chloride in the polychaete *Nereis diversicolor* in steady-state adaptation to very low salinities are reported.

2. The Na-uptake mechanism shows a high affinity for sodium, reaching half the maximal uptake rate at an external Na-concentration of 8–10 mM/liter (*ca.* 2% SW), and becomes "saturated" or reaches a plateau of uptake at concentrations of 40–50 mM/liter (*ca.* 10% SW) up to *ca.* 350 mM/liter (75% SW), above which Na-exchange is proportional to the external concentration.

3. The Cl-uptake curve differs from the Na-uptake curve in showing a relative depression at very low salinities before reaching "saturation" at Cl-concentrations of 50–60 mM/liter (*ca.* 10% SW). Cl-uptake becomes proportional to external concentration in salinities of 50% SW or greater, suggestive of passive diffusion in the ionic and osmotic conforming range.

4. It is shown that the permeability of the body wall, both to Na and to Cl, is reduced at very low salinities, thus destroying one of the assumptions upon which a previously-presented balance-sheet for chloride exchanges in *N. diversicolor* was based (Smith, 1970a).

5. Attempts to demonstrate an activation of the Na-uptake mechanism at very low salinities were inconclusive; reduction of body-wall permeability to sodium masks any possible activation.

6. It is suggested that the inside-negative body-wall potential is related to the depression of the Cl-uptake curve in salinities below 10% SW.

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